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DATA EVALUATION REPORT VIII

STUDY TYPE: Mammalian In vivo chromosomal aberrations (bone marrow cells) TOX CHEM NO.309BB(?) see note under SYNONYMS below

MRID NO: not given ACC. No. 408064-10

TEST MATERIAL: CL 182,005 (97.0%)

SYNONYMS: CL 3,4-dichloro-5-isothiazole carboxylic acid
Note: Tox. Chem. 309BB is the potassium salt of this acid.

STUDY NUMBER(S): HLA Study No. 10135-0-451

SPONSOR: American Cyanamid Company

TESTING FACILITY: Hazleton Laboratories America, Inc.
5516 Nicholson Lane, Suite 400
Kensington, MD 20895

TITLE OF REPORT: Chromosomal Aberrations in Vivo in Mammalian Bone Marrow Cells CL 182,005

AUTHOR: Ivett, J. L.

REPORT ISSUED: 05-16-88

CLASSIFICATION: Acceptable (no clastogenic activity in this assay)

CONCLUSIONS:

1. There was no evidence of an increase in incidences of chromosomal aberrations, nor was there any evidence of a consistent effect on the mitotic index in bone marrow cells from rats that were sacrificed 6, 18 or 30 hours after oral dosage at 0, 150 and 500 mg/kg or at 900 mg/kg (females only) or at 1500 mg/kg (males only).

2. The high level of mortality at 2500 mg/kg in the preliminary acute toxicity assay, as well as the relatively high mortality rates observed at 1500 mg/kg for males and at 1500 and 1250 mg/kg for females indicate the highest dosage levels were adequate.

3. The study and its findings are acceptable.

A. MATERIALS:

1. Test compound: CL 182,005, lot no. 86-58-1, 97.0% active. Described as a beige powder. "In the solubility test 2698.7 mg of CL 182,005 was suspended in 4.0 ml of 0.5% carboxymethylcellulose (CMC). This resulted in a final volume of ~5.0 ml and a final concentration of ~539.7 mg/ml. The resulting thick lumpy beige suspension would not pass through a 16 gauge intubation needle. The entire suspension was ground with a mortar and pestle. This processing resulted in an even suspension which passed through the intubation needle."

2. Positive control: Cyclophosphamide (CP, Sigma, Lot #86F-0101) at 60 mg/kg was administered by oral gavage at a volume of about 10 ml/kg.

3. Test animals: Adult male and female Sprague-Dawley rats, purchased from Charles River Breeding Laboratories, Inc. Raleigh, NC.

B. STUDY DESIGN:

1. Dose selection: "The dose levels evaluated in this dose rangefinding assay (0, 500, 1250, 2500, 3750, and 5000 mg/kg) were selected by the sponsor and were dosed at a volume of 10 ml/kg... The animals used for this trial were about 8 weeks old at the time of dosing. Six groups of animals (three per sex per group) were dosed acutely (one-time only) and were observed for toxic symptoms and/or mortalities." "Two hours prior to the scheduled sacrifice at 30 hours after dosing all surviving animals were intraperitoneally (IP) injected with 2.0 mg/kg colchicine and the bone marrow cells were subsequently harvested and processed for mitotic index analysis from 1000 bone marrow cells..."

2. Animal dosage:

There were 3 trials. In trial I dose levels were 0 (vehicle control), 150, 500 and 1500 mg/kg, based on the preliminary dose rangefinding assay. Five males and 5 females at each dose level were to be sacrificed at 6, 18

and 30 hours following dosage. Cyclophosphamide at 60 mg/kg was orally administered to a positive control group of 5 males and 5 females which were sacrificed at 18 hours post dosage.

Because of high mortality at 1500 mg/kg, there was an inadequate number of males for the 18-hour sacrifice, and there was an inadequate number of females for any sacrifice time. As a result, trial II was conducted with an 18-hr male group dosed at 1500 mg/kg, and 6, 18 and 30-hr female groups (5 animals in each) dosed at 1250 mg/kg, along with concurrent vehicle and positive control groups.

Because 13/20 females dosed at 1250 mg/kg died, trial III was conducted with 25 females orally dosed at 900 mg/kg, of which 7/25 of died. As a result there was a sufficient number of animals to be sacrificed at 6, 18 and 30 hours (5 animals sacrificed at each of these times). Again, there were concurrent vehicle and positive control groups.

3. Bone marrow harvest and slide preparation:

See appended page 1 for the procedure used for bone marrow harvest, slide preparation and analysis.

4. Evaluation criteria, data presentation and statistical evaluation:

Refer to appended pages 1 through 6 for the assay evaluation criteria, data presentation and to page 18 for the statistical evaluation.

5. There is a signed and dated Good Laboratory Practice Compliance Statement on p. 3 of the report, as well as a signed and dated Quality Assurance Statment (giving the dates of inspections) on p. 6.

C. RESULTS:

1. Acute Toxicity of CL 182,005:

Mortality:	Number dying/number dosed	
Dose (mg/kg)	Males	Females
0	0/3	0/3
500	0/3	0/3
1250	0/3	0/3
2500	2/3	2/3
3750	3/3	3/3
5000	3/3	3/3

Symptoms at the 3 highest dose levels included severe tonic

convulsions. All rats at 1250 mg/kg were lethargic and exhibited mild tonic convulsions upon tactile stimulation.

2. In vivo cytogenetic assay: There was no evidence of an increase in incidences of chromosomal aberrations, nor was there any evidence of a consistent effect on the mitotic index in any of the groups that were dosed with CL 182,005. Refer to appended pages 7 through 17 for individual animal data. The cyclophosphamide (see appended pages 7, 14 and 16) elicited appropriate positive responses.

D. DISCUSSION:

There was no detectable clastogenic activity under the conditions of this assay, at any of the dose levels, for any of the sacrifice sampling times (6, 18 and 30 hours) following dosage. The level of mortality observed at 2500 mg/kg in the preliminary acute toxicity study, as well as the relatively high mortality observed in subsequent trials for males at 1500 mg/kg and for females at 1500 and 1250 mg/kg, indicates that the high dose levels administered were adequate. Results of chemistry analyses of dosing samples were displayed within an acceptable range (see appended pages 19 through 22) in this study. The positive control, cyclophosphamide at 60 mg/kg, adequately demonstrates the sensitivity of the rat bone marrow system to detect a clastogenic effect.

The study and its negative findings are acceptable.